PILOT STUDY ON THE PREVALENCE OF *SALMONELLA* IN SLAUGHTER PIGS IN GERMANY:

III. DETECTION OF *SALMONELLAE* BY PCR, SEROVAR DISTRIBUTION, AND POPULATION ANALYSIS OF ISOLATES

HELMUTH, R.[a)], KÄSBOHRER, A.[a)], GEUE, L.[b)], RABSCH, W.[a)], PROTZ, D.[a)]

In a survey on the prevalence of Salmonellae in German slaughter pigs, almost 12,000 pig carcasses were investigated by standard cultural, immunological and PCR techniques. Seven abattoirs distributed all over Germany were included into the study. Each abattoir was investigated on ten weekly sampling occasions.

In total 6% of all slaughter pigs were positive in faecal or lymphnode material. This number increased to 10% when surface swabs were evaluated as well.

From all sources nearly 1400 bacterial isolates were obtained and characterised by traditional and molecular typing methods. Typing at the National Reference Laboratory for Salmonella (NRL) follows a hierarchic protocol. At the beginning the serotype is determined by using poly- and monoclonal antisera for standard slide agglutination. In total 28 serotypes could be detected.

Table 1 shows the distribution of the serotypes obtained and the number of weeks a particular serotype could be isolated from an individual abattoir.

Only Salmonella Typhimurium, which was the most frequently accountered serotype, could be isolated from all abattoirs. This serotype accounted for 40% of all isolates. The O5 negative variant, also called Copenhagen, was detected in 32% of the isolates. Together this S. Typhiumurium complex accounts for 72% of the isolates. The next most common serotypes S. Derby O5neg. and S. Give showed an isolation rate of 6%, followed by strains belonging to S.I. 9,12:l,v,:- and S. Enteritidis with isolation rates of 4 and 3% respectively. All other 21 Serotypes accounted for 7%.

[a)] Federal institute for health protection of consumers and veterinary medicine (BgVV), PO Box 33 00 13, D - 14191 Berlin, Germany

[b)] Federal research centre for virus diseases of animals (BFAV), Institute for epidemiological diagnostics, Wusterhausen, Germany

Table 1. Prevalence of serovars with at least two isolations in individual abbatoirs.

	Number		Abbatoir*						
Serotype	SH	Isolates	1.0	2.0	3.0	4.0	5.0	6.0	7.0
Typhimurium	7	564	10	7	7	5	7	8	2
Enteritidis	6	36		2	1	1	1	4	3
TyphimuriumO-5-	5	444	9	3	5		2	5	
Derby O-5 -	5	82	3	6	4		2	2	
London	*2*	*14*		*4*	*1*				
rough SG I	*2*	*13*					*2*	*4*	
Bovismorbificans	2	9		2	1				
Choleraesuis	2	6		1			1		
Livingstone	2	4	1					2	
Infantis	2	3					1		1
Agona	2	2					1	1	
Hadar	2	2		1					1
Total		1400	659	273	190	53	64	143	18
Number of serotypes **		28	6	12	8	4	11	14	5

SH = Number of abbatoirs with positive isolations
* Number of weeks with positive isolations
italics: Serotypes not detected by immunological test

The high incidence of S. Typhimurium made further subtyping necessary. Phagetyping, using the Collindale typing scheme developed by Anderson et al. , is used at the NRL. Table 2 shows the results obtained. With nearly 40% of all isolates DT104 predominated followed by DT193 with 16%.

However there was an uneven distribution of both serotypes among the O5 positive and negative S. Typhimurium. With almost 90% DT104 predominated in the O5 negative variants and 75% originated from surface swabs. DT193 in contrast predominated with 87% in the O5 positive serotype. Phagetype 193 was evenly distributed in faecal and lymphnode material.

The phagetype DT104 shows an increasing isolation rate in Germany since 1991 and accounts for about 30% of all contemporary S. Typhimurium isolates (Liesegang et al.). This phagetype is multiresistant (Liesegang et al., Threlfall et al.) and 91% of the isolates obtained in the survey showed resistance against tetracycline, ampicillin, chloramphenicol, sulfonamide, and streptomycin. Only 6.2% were antibiotic sensitive.

Table 2. Prevalence and distribution of the ten predominating phagetypes

Lysotype Anderson	% of Isolates N=1017	O:5+ N=540	O:5- N=432	Feces N=278	Lymph-nodes N=251	Surface swabs N=443
DT104	39,6	10,2	89,8	17,4	7,4	75,2
DT193	16,5	87,5	12,5	44,6	39,3	16,1
DT120	8,8	79,8	20,2	34,8	55,1	10,1
DT17	6,2	87,3	12,7	14,3	47,6	38,1
DT68	5,4	92,7	7,3	20,0	16,4	63,6
U302	4,5	80,4	19,6	28,3	23,9	47,8
DT12	3,6	91,9	8,1	29,7	64,9	5,4
DT170	3,0	96,8	3,2	67,7	19,4	12,9
DT186	2,0	100,0		80,0	5,0	15,0
DT107	1,5	100,0		46,7	53,3	
17 other types	8,9					

A clonal structure has been described for DT104 isolates in Great Britain (Threlfall et al.) and Germany (Liesegang et al.). This made further molecular typing necessary. Plasmid profile analysis was performed and four plasmidtypes were obtained. Type A carried the 60Md virulence plasmid only. It accounted for 28% of the isolates. Type B (19,5%) had an additional 1.4 MD plasmid. Type C (54,1%) carried the 1.4 and a 2.2 MD and the virulence plasmid. The last type was type D (4,1 %) carrying the virulence, a 4Md, 2.5Md and 1 MD plasmid.

Based on the characters (serotype, phagetype, antibiotic resistance and plasmid profile) given above a distribution of certain clones in the different abattoirs at the sampling intervals could be obtained. One abattoir seemed to have an hygienic problem with about 84% of all surface swabs being positive at week seven. The clonal analysis showed, that type C was introduced to this abattoir as a faecal contaminant at week 2 and persisted till week 9 with different isolation frequencies.

The type of analysis described above allows a detailed monitoring of the sources and ways of distribution of certain clones within the German pig production lines.

All Salmonella strains mentioned above were isolated by standard cultural techniques. However in order to gain more information on the applicability, sensitivity and reliability of PCR based detection procedures, this diagnostic technique was used on 1200 of the samples as well. The method applied was a preenrichment PCR using the *inv*A derived primers of Rahn et al.

Table 3. Detection of *Salmonella* in swine by culture and PCR methods

	Culture		
PCR	positive	negative	total
positive	28	7	35
negative	2	1027	1029
total	30	1034	1064

Sensitivity 93,3%: Probability, that culture positive samples will be detected by PCR
Specificity 99,35%: Probability, that culture negative cultures will be negative by PCR

100µl samples of the preenrichment culture were lysed after overnight incubation and subject to PCR. The 284Bp PCR product was detected in 2% Metaphor horizontal agarose gels. Table 3 shows the data obtained. With a sensitivity of 93% and a specificity of 99% it turned out, that the method used proved as a fast, sensitive and reliable screening method for *Salmonella* in various samples originating from pigs.

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